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Effects of distillers' dried grains with solubles and soybean oil on dietary lipid, fiber, and amino acid digestibility in corn-based diets fed to growing pigs

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Abstract

The use of corn co-products increases the concentration of fiber and often the use of supplemental lipids in swine diets, which may affect energy and nutrient digestibility. An experiment was conducted to determine the effects of reduced oil distillers dried grains with solubles (DDGS) and soybean oil (SBO) on dietary AA, acid hydrolyzed ether extract (AEE), and NDF digestibility in corn-based diets fed to growing pigs. Eighteen growing pigs (BW = 33.8 ± 2.2 kg) were surgically fitted with a T-cannula in the distal ileum and allocated to 1 of 6 dietary treatment groups in a 3-period incomplete Latin square design, with 9 observations per treatment. Six dietary treatments were obtained by adding 0, 20, and 40% DDGS to corn-casein diets formulated with 2 and 6% SBO. Ileal digesta and fecal samples were collected and the apparent ileal (AID) and total tract digestibility (ATTD) of AEE and NDF, and the AID of Lys were determined. Apparent values were corrected for endogenous losses of lipids and true ileal (TID) and true total tract digestibility (TTTD) values were calculated. Results showed that the AID of Lys decreased ($P < 0.001$) with the inclusion of DDGS, but was not affected ($P = 0.63$) by the inclusion of SBO. An interaction between DDGS and SBO on the AID ($P = 0.002$) and ATTD ($P = 0.009$) of NDF was observed, where the AID and ATTD of NDF decreased with DDGS at 6% SBO, but no effect was observed at 2% SBO. The AID of NDF increased with SBO at 0% DDGS, but no effect was observed at 20 or 40% DDGS. An interaction between DDGS and SBO on the AID ($P = 0.011$) and ATTD ($P = 0.008$) of AEE was observed, where the AID and ATTD of AEE increased with SBO. The AID and ATTD of AEE increased with DDGS at 2% SBO, but no effect was observed at 6% SBO. Correction by ileal and fecal endogenous loss of AEE (9.5 and 13.6 g/kg of DMI) showed that increasing dietary AEE had no effect on the TID and TTD of AEE ($P > 0.05$). In conclusion, the AID of Lys decreased with DDGS and was not affected by lipids from SBO. Greatest AID and ATTD of NDF was observed in diets with a high AEE and low NDF content. Low values of apparent digestibility of AEE in lower lipid diets are possibly the result of endogenous losses of lipids, because the true digestibility of AEE was not affected by the dietary increase of AEE.

Keywords

Distillers dried grains with solubles, soybean oil, fiber, lipid, pigs

Disciplines

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Effects of distillers dried grains with solubles and soybean oil on dietary lipid, fiber, and amino acid digestibility in corn based diets fed to growing pigs

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ABSTRACT

The use of corn co-products increases the concentration of fiber and often the use of supplemental lipids in swine diets, which may affect energy and nutrient digestibility. An experiment was conducted to determine the effects of reduced oil distillers dried grains with solubles (**DDGS**) and soybean oil (**SBO**) on dietary AA, acid hydrolyzed ether extract (**AEE**), and NDF digestibility in corn-based diets fed to growing pigs. Eighteen growing pigs (BW = 33.8 ± 2.2 kg) were surgically fitted with a T-cannula in the distal ileum and allocated to 1 of 6 dietary treatment groups in a 3-period incomplete Latin square design, with 9 observations per treatment. Six dietary treatments were obtained by adding 0, 20, and 40% DDGS to corn-casein diets formulated with 2 and 6% SBO. Ileal digesta and fecal samples were collected and the apparent ileal (**AID**) and total tract digestibility (**ATTD**) of AEE and NDF, and the AID of Lys were determined. Apparent values were corrected for endogenous losses of lipids and true ileal (**TID**) and true total tract digestibility (**TTTD**) values were calculated. Results showed that the AID of Lys decreased ($P < 0.001$) with the inclusion of DDGS, but was not affected ($P = 0.63$) by the inclusion of SBO. An interaction between DDGS and SBO on the AID ($P = 0.002$) and ATTD ($P = 0.009$) of NDF was observed, where the AID and ATTD of NDF decreased with DDGS at 6% SBO, but no effect was observed at 2% SBO. The AID of NDF increased with SBO at 0% DDGS, but no effect was observed at 20 or 40% DDGS. An interaction between DDGS and SBO on the AID ($P = 0.011$) and ATTD ($P = 0.008$) of AEE was observed, where the AID and ATTD of AEE increased with SBO. The AID and ATTD of AEE increased with DDGS at 2% SBO, but no effect was observed at 6% SBO. Correction by ileal and fecal endogenous loss of AEE (9.5 and 13.6 g/kg of DMI) showed that increasing dietary AEE had no effect on the TID and TTD of AEE ($P > 0.05$). In conclusion, the AID of Lys decreased with

DDGS and was not affected by lipids from SBO. Greatest AID and ATTD of NDF was observed in diets with a high AEE and low NDF content. Low values of apparent digestibility of AEE in lower lipid diets are possibly the result of endogenous losses of lipids, because the true digestibility of AEE was not affected by the dietary increase of AEE.

Keywords: Distillers dried grains with solubles, soybean oil, fiber, lipid, pigs

INTRODUCTION

Corn co-products are rich in dietary fiber and their inclusion in swine diets dilutes the traditionally starch-based dietary energy (Kerr et al, 2013). Dietary fiber from corn and its co-products is only partially fermented in the gastrointestinal tract of pigs because it is rich in insoluble non-starch polysaccharides, such as cellulose, arabinoxylans, and lignin (Bach Knudsen, 1997; Jaworski, 2012). Substitution of highly digestible nutrients and the limited fermentability of fiber in diets including corn co-products result in a linear decline of dietary energy digestibility (Noblet and Perez, 1993; Le Goff and Noblet, 2001; van Milgen, 2006). Technology advances in the ethanol industry have decreased the starch and lipid concentrations in distillers dried grains with solubles (**DDGS**), concentrating fiber in corn co-products, and resulting in the necessity to add greater quantities of lipids to the diet to maintain an acceptable concentration of dietary energy. Lipids in swine diets typically originate from intact lipids in feed ingredient matrices or extracted lipids such as soybean oil (**SBO**) or any number of vegetable oils (Azain, 2001; Adams and Jensen, 1984).

Extracted lipid sources induce smaller amounts of endogenous lipid losses and have greater digestibility than intact lipid sources when fed to growing pigs (Kil et al., 2010; Kim et al., 2013). Additionally, the inclusion of DDGS in swine diets decrease the apparent digestibility of dietary fiber, AA, and energy in swine diets (Urriola and Stein, 2010; Gutierrez et al., 2013). However, the effect of varying amounts of extracted lipids added to diets containing DDGS on the digestibility of fiber, lipids, and AA has not been investigated.

The objective of the present study was therefore to establish the relationships between the dietary increase in fiber and lipids from DDGS and SBO on the digestibility of AA, NDF, and acid hydrolyzed ether extract (**AEE**) in growing pigs..

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Iowa State University (12-13-7686-S).

Animals, Housing, and Experimental Design

Eighteen growing barrows (progeny of sire line 337 × dam line C-22, PIC, Hendersonville, TN) were housed in individual pens (1.2 × 1.2 m) equipped with a feeder, a cup waterer, and a half-slatted concrete floor in an environmentally controlled building. All pigs were surgically fitted with a T-cannula in the distal ileum following procedures described by Stein et al. (1998). After recovery from surgery, pigs were weighed (initial BW = 33.8 ± 2.2 kg) and randomly allocated to 1 of 6 dietary treatments groups in a 3-period incomplete Latin square design, resulting in 9 observations per treatment. Pigs were not allowed to repeat dietary treatments across periods. Each collection period involved 9 d of adaptation to dietary treatments followed by 2 d of feces sub-sample collection and 3 d of ileal digesta sub-sample collection.

Dietary Treatments

Ingredient composition of the dietary treatments is shown in Table 1. Six dietary treatments were obtained by adding 0, 20, and 40% DDGS to corn-casein diets formulated with 2% and 6% SBO. The same batch of DDGS was used for all dietary treatments and values of nutrient composition were obtained from NRC (2012). A corn-casein diet with 2% SBO was formulated to meet or exceed the nutrient requirements of growing pigs (NRC, 2012), and 5 additional dietary treatments were obtained by adding DDGS and SBO at the expense of corn. Casein and the portion of the diet including limestone, monocalcium phosphate, salt, vitamins and trace mineral premixes were maintained constant. Diets also contained 0.5% Cr_2O_3 as an inert marker. All pigs received the same daily amount of feed, which was provided at a level of approximately 90% of predicted *ad-libitum* intake of the diet formulated with 2% SBO and no DDGS. Ad-libitum intake was estimated as DE intake (kcal/d) = 13.162 × ($1-e^{-0.0176 \times BW}$), where BW was average BW (NRC, 1987). The daily feed allowance was divided into 2 equal meals provided at 0700 and 1600 h. At the end of each collection period, all pigs were weighed and daily feed allowance for the next collection period was adjusted.

Sample Collection

After 9 d of adaptation to the diet, feces were collected via grab sampling on d 10 and 11, and stored at -20°C. On d 12, 13, and 14, ileal digesta samples were collected for 8 h by attaching a 207-mL plastic bag (Whirl-Pak, Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie. Bags were removed whenever they were filled with digesta or at least every 30 min, and stored at -20°C to prevent bacterial degradation. At the conclusion of each experimental period, frozen ileal and fecal samples were allowed to thaw at room temperature and pooled within animal, with a sub-sample collected for chemical analysis. Ileal sub-samples were lyophilized before chemical analysis. Fecal sub-samples were oven-dried in a convection oven at 65°C to constant weight (Jacobs et al., 2011). Samples of diets were collected weekly, throughout the experimental period, and in the feed mill during mixing. At the conclusion of the experiment, dietary samples were pooled and sub-sampled for chemical analysis. Diets, feed ingredients, and dried ileal and fecal sub-samples were finely ground in a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ) through a 1-mm screen and stored in desiccators to maintain a constant percentage of DM.

Chemical Analysis and Calculations

Samples of diets were analyzed for DM (method 930.15; AOAC Int., 2007), GE by a bomb calorimeter (Model 6200; Parr Instrument Co., Moline, IL) with benzoic acid as a standard (6,318 kcal GE/kg of benzoic acid; Parr Instrument Co., Moline, IL), acid hydrolyzed ether extract (**AEE**; Sanderson, 1986; Soxtec 2050, FOSS North America, Eden Prairie, MN), starch (method 996.11; AOAC Int., 2007), ADF (Goering and Van Soest, 1970), neutral detergent fiber (NDF; Van Soest and Robertson, 1980), total dietary fiber (**TDF**; method 985.29; AOAC Int., 2007), and N using the combustion method (method 990.03: AOAC International, 2007) with a Trumac apparatus (Leco Corporation, St. Joseph, MI) with EDTA for calibration (9.58 ± 0.01% N; Leco Corporation, St. Joseph, MO). Crude protein was calculated as nitrogen × 6.25. Ileal digesta and fecal samples were also analyzed for DM, GE, NDF, and AEE. Diets and ileal digesta were analyzed for AA (University of Missouri Agriculture Experiment Station Chemical Laboratories, Columbia, MO) according to method 982.30 E (a, b, c; AOAC Int., 2007). Chromium was determined in diets, ileal digesta, and fecal sub-samples using the method of Fenton and Fenton (1979) and absorption was measured at 440 nm using a spectrophotometer (Synergy 4, BioTek, Winooski, VT). Chromic oxide standard samples were assayed to confirm the accuracy of the analytical procedure, and a recovery of 100.8 ± 1.95% was attained.

The DE value was determined by multiplying the GE by the observed ATTD of GE of the ingredient, and the ME was estimated from the calculated DE and CP of the ingredient

(Noblet and Perez, 1993). For each dietary treatment, the AID and ATTD of DM, GE, NDF, and AEE, and the AID of AA were calculated (Oresanya et al., 2008). True ileal digestibility (**TID**) and true total tract digestibility (**TTTD**) of AEE were calculated using the regression method (Jørgensen et al., 1993). Following the procedures outlined by Kil et al. (2010), the total apparently digested AEE (g/kg of DMI) at the end of the ileum (**$\mathbf{TAD_i}$**) and over the entire intestinal tract (**$\mathbf{TAD_t}$**) were estimated by multiplying the AID and ATTD of AEE by the dietary AEE con (g/kg of DM) of each pig, respectively. A linear response of both the TAD_i and TAD_t of AEE to the increase in dietary AEE intake (g/kg of DM) was expected, and the Y-intercepts of these two linear functions were considered to represent the estimated endogenous losses of AEE (g/kg of DMI) at the end of the ileum and over the entire intestinal tract, respectively (Jørgensen et al., 1993; Kil et al., 2010). The slopes of the linear functions are considered the average true digestibility of AEE at the ileal and total tract level. The TID and TTTD of AEE in diets were also calculated by correcting individual values of AID and ATTD for endogenous losses of AEE (g/ kg of DMI) at the end of the ileum and over the entire intestinal tract, respectively (Stein et al., 2007; Kil et al., 2010).

Statistical Analyses

Univariate Analysis and Normality Test. The data were analyzed in a mixed model (PROC MIXED, SAS Inst., Inc., Cary, NC) including the fixed effects of DDGS, SBO, their interaction and the covariate initial BW, and the random effects of Period (3 levels) and Group (6 levels with 3 pigs per group).

Studentized residuals from each analysis were and used to test normality. Outliers were removed until the Shapiro-Wilk′s test reached $P > 0.05$ and Studentized residuals fell within ±3σ.

Least squares means for DDGS and interaction between DDGS and SBO were compared using Tukey-Kramer adjustment. Effects and multiple comparison differences were deemed significant at $\alpha \leq 0.05$.

Response Surface Model. Orthogonal polynomial contrasts were used to test the linear and quadratic effects of DDGS and SBO, when possible. Linear effects tested were: DDGS (**L_DDGS**), SBO (**L_SBO**), and interaction L_SBO*L_DDGS. Quadratic effects tested were: **Q_DDGS** and the interaction L_SBO*Q_DDGS.

Depending on the significance ($P \leq 0.05$) of the contrasts, response surface models were generated to predict response according to the dietary concentrations of DDGS and SBO. A hierarchical model selection was used to construct the response surface models. Models were only constructed when both DDGS and SBO were significant ($P \leq 0.05$), regardless of the orthogonal polynomial effect (linear or quadratic). If the quadratic effect of Q_DDGS was significant ($P \leq 0.05$), the linear effect of L_DDGS was kept in the model. Similarly, if the interaction L_SBO*Q_DDGS was significant ($P \leq 0.05$), the interaction L_SBO*L_DDGS was kept in the model, as well as all other effects following the hierarchy of the model (L_SBO, L_DDGS, and Q_DDGS), since these effects are part of the interaction effect of L_SBO*Q_DDGS. Since SBO had only two levels (2 and 6%), we could only test the linear effect (L_SBO and interactions).

Estimation of endogenous losses of lipids. In addition to the analyses of the raw data for AID and ATTD of AEE, these traits were adjusted for the estimated endogenous losses of AEE and reanalyzed as TID and TTTD. Estimation of the endogenous loss of AEE for each lipid trait was performed using a linear regression of the ileal and fecal TAD AEE on the AEE intake from the 6 dietary treatments. The 6 dietary treatments represent the combination of the 2 SBO and 3 DDGS. The intercept of the ileal and fecal models was considered the endogenous loss of AEE at each compartment, respectively. The TID and TTTD of AEE were then estimated as:

$$Y_{ij} = \frac{(TAD_{ij} - endogenous\ loss\ of\ AEE)}{intake\ of\ AEE_j}$$

where: Y_{ij} is the estimated TID or TTTD of AEE of the i^{th} observation in the j^{th} dietary treatment (j=1 to 6), TAD_{ij} is the observed value of TADi or TADt AEE, and intake of AEE_j is the intake of AEE of the j^{th} dietary treatment. The estimated TID and TTTD of AEE were analyzed using the same mixed model previously described. All statistical analyses were performed using SAS 9.3 (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

All pigs successfully recovered from surgery, remained healthy and readily consumed their diets throughout the experiment. The NDF and AEE of the DDGS used in the present study were 39.2% and 7.1%, respectively. The analyzed nutrient composition of diets showed that dietary inclusion of DDGS increased the dietary AEE in treatments formulated with 2% SBO (4.4, 5.7, and 6.3% AEE) and 6% SBO (8.4, 9.1, and 10.1% AEE: Table 2). The dietary NDF also increased with DDGS in treatments formulated with 2% SBO (6.9, 11.0, and 14.6% NDF) and 6% SBO (6.8, 9.7, and 14% NDF).

Apparent Digestibility of Energy and Amino Acids

The AID and ATTD of GE and DM decreased with dietary inclusion of DDGS ($P < 0.001$; Table 3), resulting in DE and ME values of diets formulated with 2% and 6% SBO decreasing ($P < 0.01$) with DDGS addition (Table 4). The decrease in dietary energy is a direct result of gradually replacing highly digestible starch with low-digestible dietary fiber from DDGS. In growing pigs it has been previously estimated that % increase in dietary NDF reduced the energy digestibility by 0.9% (Le Goff and Noblet, 2001). Similar effects on apparent digestibility of DM have been previously reported after insoluble dietary fiber was included at the expense of a highly digestible source of carbohydrates (Schulze et al., 1994; Wilfart et al., 2007). In contrast, the dietary inclusion of SBO increased the AID of GE ($P = 0.046$), resulting in DE and ME values of diets increasing ($P < 0.01$) by approximately 0.2 Mcal/kg of ME when SBO increased from 2% to 6% in the diet. The large effect of adding DDGS and SBO on the apparent digestibility of DM, GE, and on DE and ME values is supported by the response surface models (Table 5), where a high predictability of AID of GE ($R^2 = 0.78$), DM ($R^2 = 0.86$), DE ($R^2 = 0.83$), and ME ($R^2 = 0.87$) from SBO and DDGS inclusion was observed.

The AID of all indispensible AA decreased ($P < 0.05$) with DDGS and, with the exception of the AID of Ile ($P = 0.015$) and Met ($P = 0.008$), were not affected by SBO ($P > 0.05$). A declines in the AID of AA from the dietary increase of co-products from the corn-ethanol distillation industry has also been previously reported (Urriola and Stein, 2010; Gutierrez et al., 2013). The decrease in AID of AA observed may result from the combination of an increase in the amount of endogenous N loss and a decrease in absorption of endogenous and exogenous N. Dietary fiber intake has been associated with the increase of endogenous loss of nitrogen (Souffrant, 1991, 2001; Moughan, 2003), and insoluble fiber in particular associated with the secretion of pancreatic juice (Langlois et al., 1987). Although the increase of dietary fiber may reduce the digestibility of AA (Schulze et al., 1994), insoluble dietary fiber, such as corn fiber, has been reported to have only minor effects on the digestibility of dietary AA (Zhu et al., 2005) and on the basal endogenous losses of AA (Leterme et al., 1996). Therefore, the formation of biologically unavailable Maillard reactions products from excessive heating, and the addition of solubles, is likely the cause of the lower AID of Lys in DDGS (Pahm et al., 2008; Stein and Shurson, 2009).

Apparent Digestibility of Fiber

An interaction between DDGS and SBO on the AID ($P = 0.002$) and ATTD ($P = 0.009$) of NDF was observed. The dietary increase of DDGS at 2% SBO, and the subsequent increase in dietary insoluble NDF, showed no effect on the apparent digestibility of NDF at the terminal ileum and over the entire intestinal tract (Figure 1). Similar results on the AID of insoluble dietary fiber have been previously reported (Graham et al., 1986; Urriola and Stein, 2010; Gutierrez et al., 2013), and AID of NDF values observed in the present study (≈ 28% AID of NDF at 2% SBO) agree with previously reported data from corn bran (Gutierrez et al., 2013). Fermentation of dietary fiber at the terminal ileum is well documented, and the extent varies with the solubility of dietary fiber (Jørgensen et al., 1996; Noblet and Le Goff, 2001; Urriola and Stein, 2010; Gutierrez et al., 2013). Surprisingly, the ATTD of NDF was not affected by DDGS increase in diets with 2% SBO, which differ from previous reports where the increase in insoluble dietary fiber from corn origin decreased the ATTD of dietary fiber (Urriola and Stein, 2010; Gutierrez et al., 2013).

Conversely, the dietary increase of DDGS in diets with 6% SBO, and the subsequent increase in dietary insoluble NDF, resulted in a reduction of the ileal and fecal apparent digestibility of NDF. The different response to DDGS addition between diets with 2% and 6%

SBO is possibly the consequence of the elevated dietary lipid concentration in the latter set of diets, and the ability of dietary lipids to increase the retention time of digesta in low fiber diets. Dietary lipids have been previously reported to increase the retention time of digesta in pigs (Cervantes-Pahm and Stein, 2008) and in laying hens (Mateos et al., 1982). An increase in the retention time of digesta may also increase the fermentability of fiber due to a longer period of exposure of substrates to the intestinal microbiota at the terminal ileum or in the hindgut (Morel et al., 2006; Wilfart et al., 2007). This effect may be noticeable at 0% DDGS and 6% SBO, where dietary NDF is low but AEE is high, and where the AID and ATTD of NDF were the greatest (46.4% and 52.0%, respectively). It is also evident when the AID and ATTD of NDF increased with the increase of SBO in diets with the lowest fiber content (0% DDGS).

The possibility of a longer transit time in pigs fed diets with 6% SBO may be offset by the dietary increase of NDF from DDGS, decreasing in turn the apparent digestibility of NDF. A dietary increase of NDF from DDGS may stimulate bowel movement and reduce the transit time of digesta (Bastianelli et al., 1996; Schneeman, 1998; Bindelle et al., 2008), which agree with data from pigs fed insoluble dietary fiber from wheat bran where a decrease in retention time of digesta was reported (Wilfart et al., 2007). The moderate predictability of AID and ATTD of NDF ($R^2 = 0.53$ and $R^2 = 0.36$, respectively) from SBO and DDGS may provide evidence the difficulty of the interaction between a fibrous and lipid-rich ingredients.

Values of apparent digestibility of NDF were greater over the entire intestinal tract than at the terminal ileum. This observation suggests that part of fiber from corn origin is fermented at the terminal ileum and an additional portion is fermented in the hindgut of pigs (Urriola and Stein, 2010; Gutierrez et al., 2013). The value of ATTD for dietary fiber is therefore an accurate estimate of the total fermentation of dietary fiber in pigs.

Apparent Digestibility of Acid Hydrolyzed Ether Extract

The dietary AEE increased with SBO and DDGS inclusion. An interaction between DDGS and SBO on the AID (P = 0.011) and ATTD (P = 0.008) of AEE was observed, where the AID and ATTD of AEE increased with the dietary level of AEE from SBO, and increased likewise with the dietary level of AEE from DDGS but only when added to 2% SBO diets (Table 6). An interaction between DDGS and SBO on the TAD_i (P = 0.001) and TAD_t (P = 0.001) was also observed, where the TAD_i and TAD_f increased with DDGS at the two levels of SBO, but values were greater at 6% SBO than at 2% SBO. A similar interaction between dietary lipid and fiber concentrations on the apparent digestibility of dietary lipids has also been previously reported (Dégen et al., 2009), and the positive relationship between dietary increase and apparent digestibility of AEE observed in the present study agree with previously reported data from pigs (Just et al., 1980; Jørgensen et al., 1993; Kim et al., 2013).

The observed response in apparent digestibility of AEE was modulated by the source of dietary lipids. This was evidenced by the response surface models, where the magnitude of the slopes for the effects of SBO on AID and ATTD of AEE were greater than those of DDGS. This observation is supported by the high predictability of the AID and ATTD of AEE (R^2 = 0.68 and R^2 = 0.79, respectively) from DDGS and SBO inclusion (Figure 2). The shape of the observed response originate from the fact that apparent digestibility of extracted lipid is greater than for intact lipids, as previously reported in pigs fed diets supplemented with SBO (Agunbiade et al., 1992), palm kernel oil (Agunbiade et al., 1999), sunflower oil (San Juan and Villamide, 2000), and corn oil (Kim et al., 2013). Therefore, greater values of AID and ATTD of AEE were observed in diets formulated with 6% SBO, where most of the AEE content is extracted lipids, than in diets formulated with 2% SBO, where most of the AEE content is intact lipids. Values of

AID and ATTD of AEE in the present study fall within the range of values reported by Kil et al. (2010) for diets formulated with extracted or intact lipids.

However, low apparent digestibility values for AEE from diets formulated with low levels of dietary AEE may result from the contribution of endogenous losses of AEE to the total AEE output (Jørgensen et al., 1993; Kil et al., 2010; Kim et al., 2013). Similar effects of endogenous losses on the apparent digestibility of dietary AA from have also been reported previously (Fan and Sauer, 1997).

The increase of dietary fiber intake may stimulate the synthesis of endogenous microbial lipids in the hindgut, increasing in turn the endogenous losses of lipids at the total tract level and reducing the ATTD of lipids. A net synthesis of lipids in the hindgut of pigs has been previously reported (Shi and Noblet, 1993; Bakker, 1996). Kil et al. (2010) reported greater values of AID vs. ATTD of AEE from semi-purified diets formulated with increasing levels of fiber from Solka-Floc. These results suggest that dietary lipids are digested and absorbed before the end of the ileum, and that differences between values of AID and ATTD of AEE are the product of endogenous microbial lipid synthesis in the hindgut of pigs (Low, 1980; Drackley, 2000). Corn and DDGS were the only fiber sources in the present study. Fiber from corn origin is fermented partially because of its chemical composition; a small portion is fermented by microbes at the ileal level, and the majority of corn dietary fiber reaches the hindgut where is moderately fermented (Gutierrez et al., 2013). The increase in corn fiber reaching the hindgut may stimulate microbial activity and lipid synthesis (Eyssen, 1973; Bach Knudsen et al., 1991), increasing endogenous losses of lipids from microbial origin, which may explain the lower values in ATTD of AEE relative to AID observed in the present study. Values of AID of AEE therefore reflect more accurately the digestibility of dietary AEE than ATTD values.

In the present study, dietary NDF increased similarly with inclusion of DDGS in diets containing 2% or 6% SBO. A decrease in the apparent digestibility of lipids with a dietary increase of NDF has been previously reported (Just et al., 1980; Bakker, 1996; Hansen et al., 2006), but the reduction has been associated with characteristics of dietary fiber such as solubility and viscosity, rather than its dietary concentration (Fahey et al., 1990; Bach Knudsen and Hansen, 1991; Smits and Annison, 1996). Fiber in corn and its co-products is highly insoluble and has low viscosity (Jaworski, 2012; Gutierrez et al., 2014); therefore, the increase of dietary fiber from the dietary inclusion of DDGS may not negatively affect the apparent digestibility of AEE at 2% or 6% SBO. This assumption is supported by Kil et al. (2010), who observed no effects of dietary increase of highly insoluble and low viscous purified NDF on the apparent digestibility of AEE.

Endogenous Losses and True Digestibility of Acid Hydrolyzed Ether Extract

The TAD_i and TAD_t of AEE increased ($P < 0.01$) with AEE intake (Figure 3), and the estimated linear functions explained 99 % of the variation in TAD_i and TAD_t. Linearity is a prerequisite of the linear regression method, and the observed linear response supports its use for the estimation of endogenous loss of AEE from both compartments of the digestive system (Jørgensen et al., 1993; Fan and Sauer, 1997). The endogenous losses of AEE, corresponding to the *y*-intercepts of the linear functions, were 9.47 g/kg of DMI at the end of the ileum and 13.64 g/kg of DMI over the entire intestinal tract. The average true digestibility of AEE at the end of the ileum and over the entire intestinal tract, estimated from the slope of the linear functions, was 94 % and 93 %, respectively. The TID and TTTD of AEE (Table 7), calculated by adjusting the AID and ATTD for endogenous losses, showed that increasing the dietary inclusion of SBO or DDGS had no effect ($P > 0.05$) on TID or TTTD.

The source of dietary lipids may result in different estimates of endogenous lipid loss at both the ileal and total tract levels. Estimates of endogenous losses of AEE from feeding intact lipids reported by Kil et al. (2010) were greater than estimates from extracted lipids, at both the ileal and total tract levels. Greater endogenous loss of AEE from feeding intact lipids may result from the commonly greater concentrations of fiber in ingredients containing intact lipids. Fiber in the feed ingredient may depress absorption of dietary lipids and resorption of endogenous lipids, resulting in greater endogenous lipid loss (Bach Knudsen and Hansen, 1991). Dietary treatments in the present study were formulated with varying ratios of intact to extracted lipids; therefore, estimates of endogenous losses of AEE for these diets are not directly comparable to estimates reported previously for intact or extracted lipid sources. Additionally, discrepancies between estimates of endogenous lipid loss may result from the fact that the range in dietary lipid concentrations differed among experiments. Although pigs in the present study were not offered diets with very low AEE levels, the regression curves calculated were able to explain approximately 99% of the variation in totally apparently digested AEE. Other factors such analytical method to estimate lipid concentrations (ether extract vs. AEE), lipid sources (Kim et al., 2013), non-lipid dietary components (Jørgensen et al., 1992b), and animal effects (Jørgensen et al., 2012a) may also affect the estimates of endogenous lipid loss.

Greater true digestibility of extracted lipids compared with intact lipids at both collection sites has been reported previously (Kil et al., 2010). This observation results from the fact that intact lipids are encased within cell membranes, therefore are more resistant to the formation of emulsions and enzymatic digestion than extracted sources (Adams and Jensen, 1984; Bach Knudsen et al., 1993). The average TID (94.2%) and TTTD (93.1%) of AEE estimated in this experiment are similar to values for extracted lipids (93.8 and 94.2%, respectively) reported by

Kil et al. (2010). The average TTTD value also falls within the range of values (91.2 to 97.7%) reported for the TTTD of SBO (Adams and Jensen, 1984; Jørgensen et al., 1993; Jørgensen and Fernández, 2000). The similarity between estimates of true digestibility in the present study and previous estimates for extracted lipids may rely on the fact that SBO was an important contributor to the total lipid content in experimental diets, ranging from approximately 30% to 67%. Additionally, an important portion of the lipids in DDGS has been previously extracted from the corn grain and added back in the solubles prior to drying (Díaz-Royón et al., 2012).Its digestion should therefore be similar to an extracted source. As outlined by Kil et al. (2010), additional variation in TID and TTD for AEE among experiments can be introduced because of differences in dietary lipid intake, diet composition, diet processing, and lipid sources.

Although the apparent digestibility of AEE increased with the inclusion of dietary AEE from SBO and DDGS, the true digestibility of AEE was not affected by the dietary concentration of AEE. The reason for this observation is that the endogenous loss of AEE affects the apparent digestibility, but not the true digestibility of AEE. These results therefore support the hypothesis that the true digestibility of a lipid source is not affected by the amount of dietary lipid intake (Freeman et al., 1968; Adams and Jensen, 1984).

Conclusions

Results from the present experiment showed that the AID of AA was negatively associated with the concentration of DDGS. No effect on the AID and ATTD of NDF from the dietary increase of NDF from DDGS was observed in diets with low concentrations of AEE from SBO. However, in diets with high concentrations of AEE from SBO, and low NDF from DDGS, the AID and ATTD of NDF was greatest. The high concentration of AEE, and low concentration of NDF, may increase the retention time of digesta and therefore the fermentability of NDF in the gastrointestinal tract. The AID and ATTD of AEE increased with dietary AEE inclusion from SBO and DDGS. Low values of apparent digestibility of AEE in diets with low concentrations of AEE are possibly the result of endogenous losses of AEE, because the true digestibility of AEE was not affected by the dietary increase of AEE.

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Table 1. Ingredient composition (%) of the experimental diets (as-fed basis)[1]

SBO, %	2			6		
Item DDGS, %	0	20	40	0	20	40
Ingredient, %						
Corn	81.7	61.7	41.7	77.7	57.7	37.7
DDGS	0.0	20.0	40.0	0.0	20.0	40.0
Casein	12.5	12.5	12.5	12.5	12.5	12.5
Soybean oil	2.0	2.0	2.0	6.0	6.0	6.0
Limestone	1.3	1.3	1.3	1.3	1.3	1.3
Monocalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[2]	0.2	0.2	0.2	0.2	0.2	0.2
Trace mineral premix[3]	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.6	0.6	0.6	0.6	0.6	0.6
Energy and nutrients[4]						
ME, Mcal/kg	3.39	3.33	3.27	3.59	3.54	3.48
SID Lys, %	0.98	1.03	1.08	0.98	1.02	1.07
SID Thr, %	0.61	0.71	0.80	0.61	0.70	0.80
SID Met+Cys, %	0.60	0.70	0.80	0.59	0.69	0.78
SID Trp, %	0.20	0.22	0.23	0.20	0.21	0.23

[1]SBO = soybean oil; DDGS = reduced oil distillers dried grains with solubles.

[2]Provided per kilogram of complete diet: 6,614 IU of vitamin A; 827 IU of vitamin D; 26 IU of vitamin E; 2.6 mg of vitamin K; 29.8 mg of niacin; 16.5 mg of pantothenic acid; 5.0 mg of

riboflavin; 0.023 mg of vitamin B_{12}.

[3]Provided per kilogram of complete diet: Zn, 165 mg as $ZnSO_4$; Fe, 165 mg as $FeSO_4$; Mn, 39 mg as $MnSO_4$; Cu, 17 mg as $CuSO_4$; I, 0.3 mg as $Ca(IO_3)_2$; and Se, 0.3 mg as Na_2SeO_3.

[4]Values were calculated (NRC, 2012). SID = standardized ileal digestible.

Table 2. Analyzed nutrient composition of the experimental diets (% as-fed basis)[1]

SBO, %	2			6				
Item DDGS, %	0	20	40	0	20	40	DDGS	SBO
DM, %	89.1	88.0	89.5	89.2	89.8	89.5	89.8	-
GE, Mcal/kg	3.98	4.08	4.18	4.18	4.33	4.42	4.40	9.16
AEE, %	4.4	5.7	6.3	8.4	9.1	10.1	7.1	98.1
Starch, %	51.1	39.2	27.2	48.6	36.7	24.7	2.9	-
ADF, %	2.4	3.4	4.6	2.2	3.4	4.5	15.8	-
NDF, %	6.9	11.0	14.6	6.8	9.7	14.0	33.8	-
CP	17.9	21.8	25.7	17.5	21.4	25.4	26.7	-
Indispensable AA								
Arg	0.7	0.8	1.0	0.6	0.8	1.0	1.3	-
His	0.5	0.6	0.8	0.5	0.6	0.8	0.9	-
Ile	0.9	1.0	1.2	0.9	1.0	1.2	1.1	-
Leu	2.0	2.3	2.8	1.9	2.3	2.9	3.3	-
Lys	1.2	1.3	1.4	1.1	1.2	1.5	1.0	-
Met	0.5	0.5	0.6	0.5	0.5	0.6	0.6	-
Phe	1.0	1.1	1.3	0.9	1.1	1.3	1.4	-
Thr	0.8	0.9	1.0	0.7	0.9	1.1	1.1	-
Trp	0.2	0.2	0.3	0.2	0.2	0.3	0.2	-
Val	1.1	1.3	1.5	1.1	1.2	1.5	1.4	-
Dispensable AA[2]								
Mean	10.1	11.6	13.3	11.2	13.5	10.1	1.1	-

All AA[3]	18.9	21.7	25.1	21.0	25.6	18.9	1.2	-

[1]SBO = soybean oil; DDGS = distillers dried grains with solubles; AEE = acid hydrolyzed ether extract; TDF = total dietary fiber.

[2]Sum of Ala, Asp, Cys, Glu, Gly, Pro, Ser, and Tyr.

[3]Sum of all indispensable and dispensable AA.

Table 3. Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of components of experimental diets[1,2]

	SBO, %	2			6				*P*-value		
Item	DDGS, %	0	20	40	0	20	40	pSEM	SBO	DDGS	SBO x DDGS
AID, %											
GE		80.6[a]	73.3[cb]	70.0[c]	84.0[a]	74.3[b]	70.1[c]	0.96	0.046	<0.001	0.178
DM		80.2[a]	71.1[b]	66.1[c]	83.4[a]	71.4[b]	65.0[c]	0.96	0.325	<0.001	0.076
NDF		32.5[b]	26.8[b]	25.3[b]	46.4[a]	21.7[b]	22.4[b]	3.12	0.389	<0.001	0.002
ATTD, %											
GE		87.8[a]	84.0[b]	80.0[c]	88.3[a]	84.3[b]	80.0[c]	0.48	0.509	<0.001	0.813
DM		88.3[a]	83.8[b]	79.0[c]	88.7[a]	83.9[b]	78.3[c]	0.46	0.892	<0.001	0.424
NDF		47.3[ab]	48.7[ab]	45.5[bc]	52.0[a]	45.7[abc]	40.9[c]	2.28	0.451	<0.001	0.009
AID of AA, %											
Arg		84.2[ab]	81.9[b]	83.2[b]	87.0[a]	82.4[b]	83.2[ab]	1.06	0.061	0.001	0.145
His		87.1[a]	82.8[b]	83.1[b]	89.5[a]	82.6[b]	83.1[b]	0.99	0.160	<0.001	0.107
Ile		87.6[a]	82.5[b]	81.8[b]	88.9[a]	82.9[b]	83.3[b]	0.67	0.015	<0.001	0.539
Leu		90.1[a]	87.0[b]	87.5[b]	91.3[a]	86.9[b]	87.7[b]	0.73	0.239	<0.001	0.435

Lys	88.9[a]	85.2[b]	84.6[b]	90.2[a]	84.3[b]	84.7[b]	0.82	0.629	<0.001	0.127
Met	92.8[b]	90.0[c]	89.6[c]	93.7[a]	90.1[c]	90.1[c]	0.44	0.008	<0.001	0.223
Phe	90.1[a]	86.9[b]	87.3[b]	91.2[a]	86.6[b]	87.3[b]	0.79	0.588	<0.001	0.390
Thr	81.4[a]	75.4[b]	74.6[b]	82.8[a]	74.7[b]	75.6[b]	1.18	0.371	<0.001	0.434
Trp	89.2[a]	84.6[b]	84.4[b]	90.7[a]	83.7[b]	84.4[b]	1.04	0.754	<0.001	0.242
Val	86.7[a]	81.7[b]	81.7[b]	87.9[a]	81.8[b]	82.4[b]	0.86	0.191	<0.001	0.665
Indispensable[3]	88.1[a]	84.0[b]	84.1[b]	89.4[a]	83.9[b]	84.4[b]	0.81	0.253	<0.001	0.388
Dispensable[4]	84.9[a]	80.9[b]	80.0[b]	87.5[a]	81.0[b]	80.8[b]	1.02	0.052	<0.001	0.256
All AA[5]	86.4[a]	82.4[b]	82.0[b]	88.4[a]	82.4[b]	82.5[b]	0.88	0.096	<0.001	0.274

pSEM = Pooled SEM

[a,b,c,d] Means within a row lacking a common superscript letter are different ($P \leq 0.05$).

[1]Least squares means of 9 pigs per diet.

[2]SBO = soybean oil; DDGS = reduced oil distillers dried grains with solubles; AEE = acid hydrolyzed ether extract.

[3]Average AID for all indispensable AA.

[4]Average AID for all dispensable AA.

[5]Average AID for all AA (indispensable and dispensable).

Table 4. Estimated digestible and metabolizable energy value of diets[1,2]

	SBO, %	2			6				*P*-value		
Item	DDGS-RO, %	0	20	40	0	20	40	pSEM	SBO	DDGS-RO	SBO*DDGS-RO
As-fed basis, Mcal/kg											
	DE	3.49^{cb}	3.43^{c}	3.34^{d}	3.69^{a}	3.65^{a}	3.53^{b}	0.020	<0.001	<0.001	0.639
	ME	3.37^{b}	3.28^{c}	3.17^{d}	3.57^{a}	3.49^{a}	3.35^{cb}	0.019	<0.001	<0.001	0.617

pSEM = Pooled SEM

[a,b,c]Means within a row lacking a common superscript letter are different ($P \leq 0.05$).

[1] Least squares means of 9 pigs per diet.

[2]SBO = soybean oil; DDGS-RO = reduced oil distillers dried grains with solubles.

Table 5. Regression coefficients of the response surface models for the effects of soybean oil (SBO) and reduced oil distillers dried grains with solubles (DDGS) on apparent digestibility and energy value of diets[1,2]

	Regression components						
Trait	Intercept	SBO	DDGS	$DDGS^2$	SBO*DDGS	$SBO*DDGS^2$	R^2
AID							
GE	81.2908	0.3100	-0.5447	0.0057	-	-	0.78
		(0.046)	(<0.001)	(0.005)	(0.071)	(0.651)	
DM	78.9176	0.7571	-0.5133	0.0056	-0.0318	-	0.86
		(0.325)	(<0.001)	(0.005)	(0.026)	(0.629)	
NDF	24.4572	3.7063	0.5498	-0.0118	-0.3960	0.0069	0.53
		(0.389)	(<0.001)	(0.005)	(0.003)	(0.037)	
AEE	67.2575	2.7325	0.2469	$-5*10^{-5}$	-0.0425	-	0.68
		(<0.001)	(0.001)	(0.894)	(0.003)	(0.800)	
ATTD							
NDF	44.8490	1.2009	0.2205	-0.0026	-0.0722	-	0.36
		(0.451)	(<0.001)	(0.571)	(0.004)	(0.256)	

AEE	55.3292	3.5898	0.3654	-0.0002	-0.0512	-	0.79
		(<0.001)	(<0.001)	(0.413)	(0.004)	(0.153)	
AID of AA							
Met	93.0344	0.0949	-0.2458	0.0039	-	-	0.71
		(0.008)	(<0.001)	(<0.001)	(0.442)	(0.111)	
Energy concentration, as-fed basis							
DE	3.4098	0.0483	-0.0041	-	-	-	0.83
		(<0.001)	(<0.001)	(0.123)	(0.745)	(0.384)	
ME	3.2922	0.0469	-0.0053	-	-	-	0.87
		(<0.001)	(<0.001)	(0.133)	(0.670)	(0.387)	

[1]Traits included were simultaneously significant for SBO and DDGS (linear or quadratic) or any of the possible interactions.

[2]*P*-values in brackets.

Table 6. Apparent digestibility and total apparently digested acid hydrolyzed ether extract (AEE) at the end of the ileum and over the entire intestinal tract of diets containing extracted or intact lipids[1,2]

	SBO, %	2			6						
	DDGS, %	0	20	40	0	20	40		*P*-value		
Item	AEE intake, g/kg of DM	44.2	57.3	63.3	84.2	90.9	100.9	pSEM	SBO	DDGS	SBO*DDGS
AID of AEE, %		72.5^{c}	75.9^{bc}	79.1^{b}	83.5^{a}	83.4^{a}	83.9^{a}	1.05	<0.001	0.005	0.011
TAD_i AEE, g/kg of DMI		32.0^{f}	43.3^{e}	49.7^{d}	70.4^{c}	75.6^{b}	84.4^{a}	0.72	<0.001	<0.001	0.001
ATTD of AEE, %		62.6^{d}	67.6^{c}	71.6^{b}	77.5^{a}	77.0^{a}	80.4^{a}	1.30	<0.001	<0.001	0.008
TAD_t AEE, g/kg of DMI		27.8^{f}	38.5^{e}	45.0^{d}	65.2^{c}	69.9^{b}	80.9^{a}	0.89	<0.001	<0.001	0.001

pSEM = Pooled SEM

[a,b,c]Means within a row lacking a common superscript letter are different ($P \leq 0.05$).

[1] Least squares means of 9 pigs per diet.

[2]SBO = soybean oil; DDGS = reduced oil distillers dried grains with solubles; AID = apparent ileal digestibility; ATTD = apparent total tract digestibility. TAD_i = total apparently digested at the end of the ileum; TAD_t = total apparently digested over the entire intestinal tract.

Table 7. True ileal digestibility (TID) and true total tract digestibility (TTTD) of acid hydrolyzed ether extract (AEE) of diets containing extracted or intact lipids[1,2]

	SBO, %	2			6				*P*-value		
Item	DDGS, %	0	20	40	0	20	40	pSEM	SBO	DDGS	SBO*DDGS
TID of AEE, %		94.0	92.5	94.1	94.8	93.8	93.2	1.06	0.586	0.467	0.506
TTTD of AEE, %		93.6	91.5	93.2	93.7	92.0	93.9	1.31	0.587	0.108	0.959

pSEM, Pooled SEM

[a,b,c]Means within a row lacking a common superscript letter are different ($P \leq 0.05$).

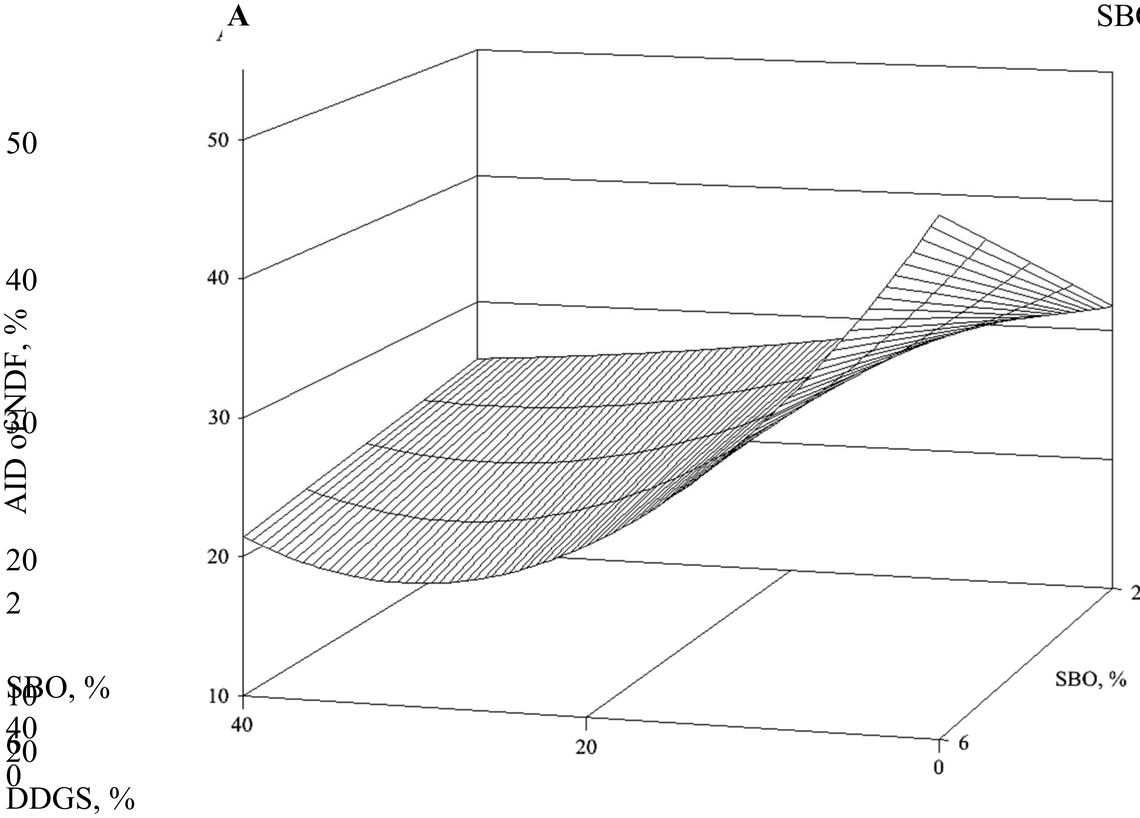
[1] Least squares means of 9 pigs per diet.

[2]SBO = soybean oil; DDGS = reduced oil distillers dried grains with solubles.

A SBO × DDGS^2

$P = 0.037$

$R^2 = 0.53$



B SBO × DDGS

$P = 0.004$

$R^2 = 0.36$

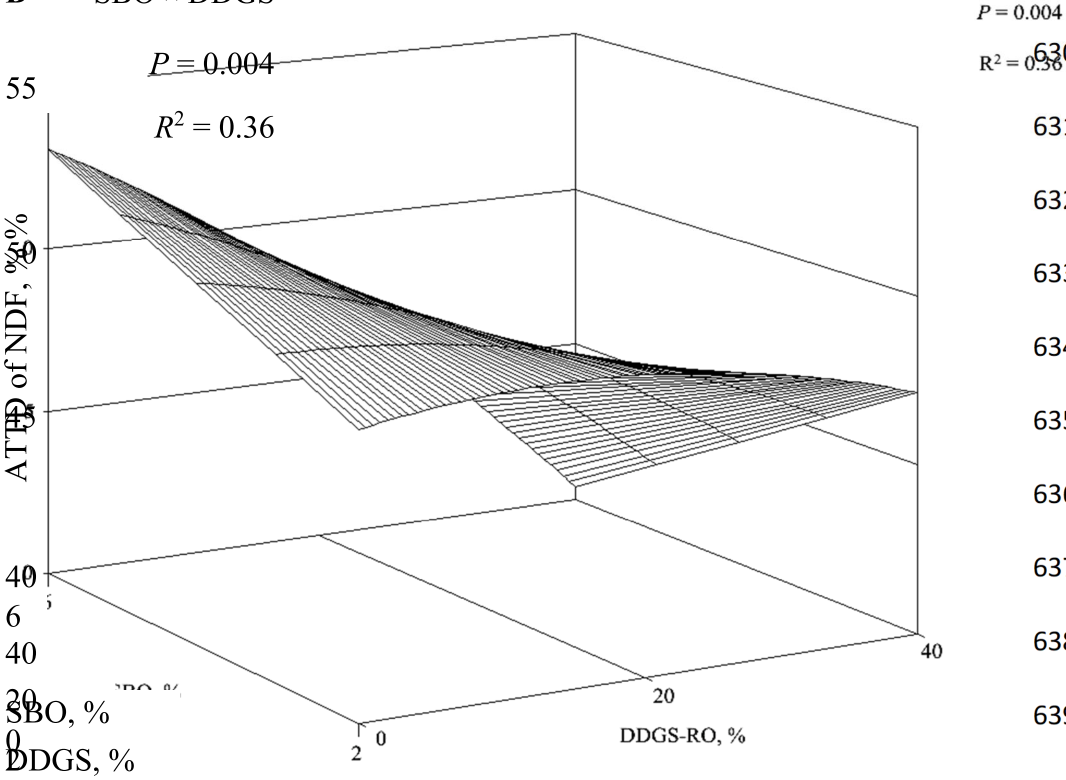


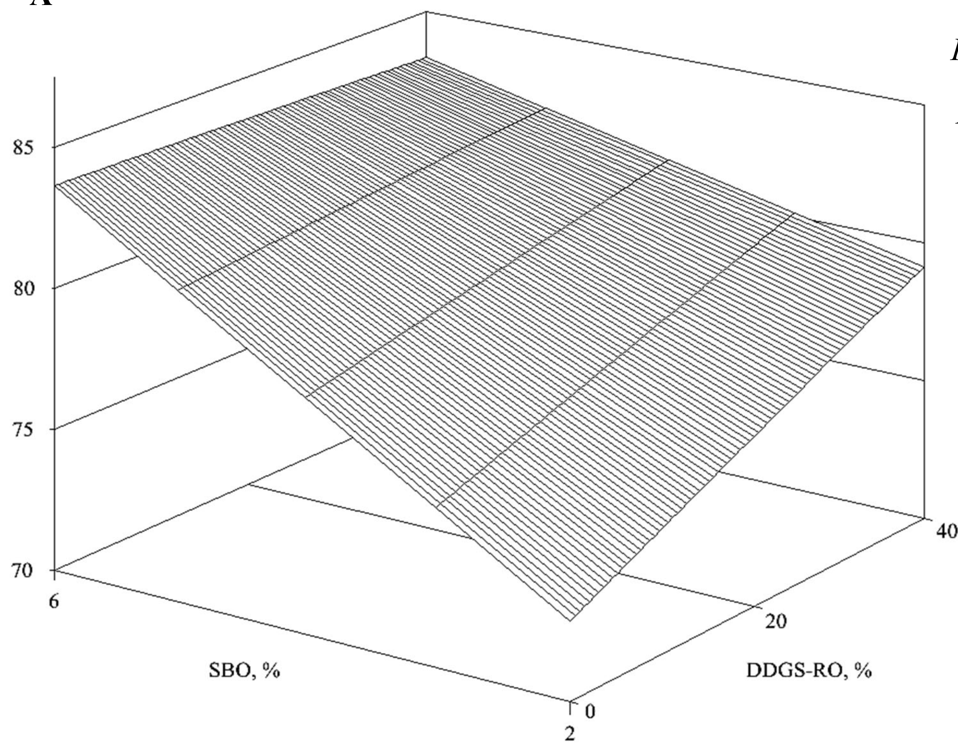
Fig. 1. Response surface for the effects of the dietary soybean oil (SBO) and reduced oil distillers dried grains with solubles (DDGS) on the apparent ileal (AID; A) and total tract digestibility (ATTD; B) of NDF in growing pigs (n = 9).

A

SBO × DDGS

$P = 0.003$

$R^2 = 0.68$



85

AID of AEE%

80

75

40

70

6

20

SBO, %

DDGS, %

0

2

A

663

665

666

667

668

669

670

671

672

673

674

SBO × DDGS

$P = 0.004$

$R^2 = 0.79$

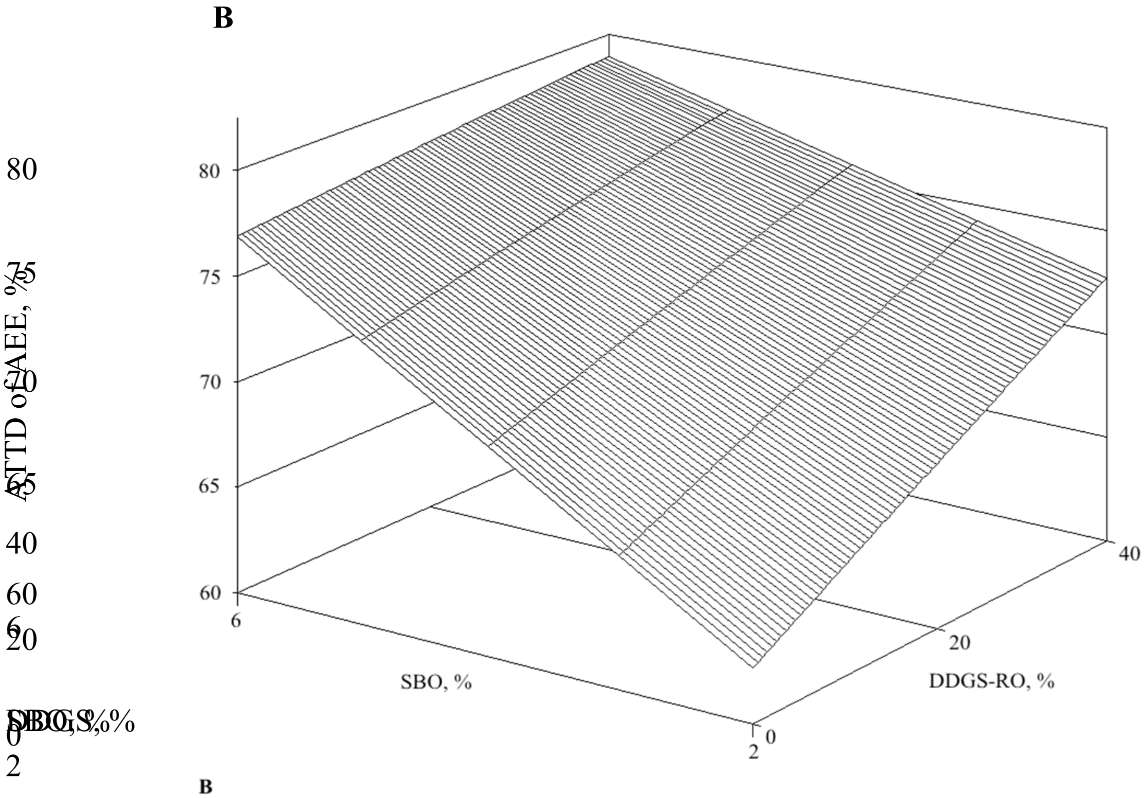


Fig. 2.

Response surface for the effects of the dietary soybean oil (SBO) and reduced oil distillers dried grains with solubles (DDGS) on the apparent ileal (AID; A) and total tract digestibility (ATTD; B) of acid hydrolyzed ether extract (AEE) in growing pigs (n = 9).

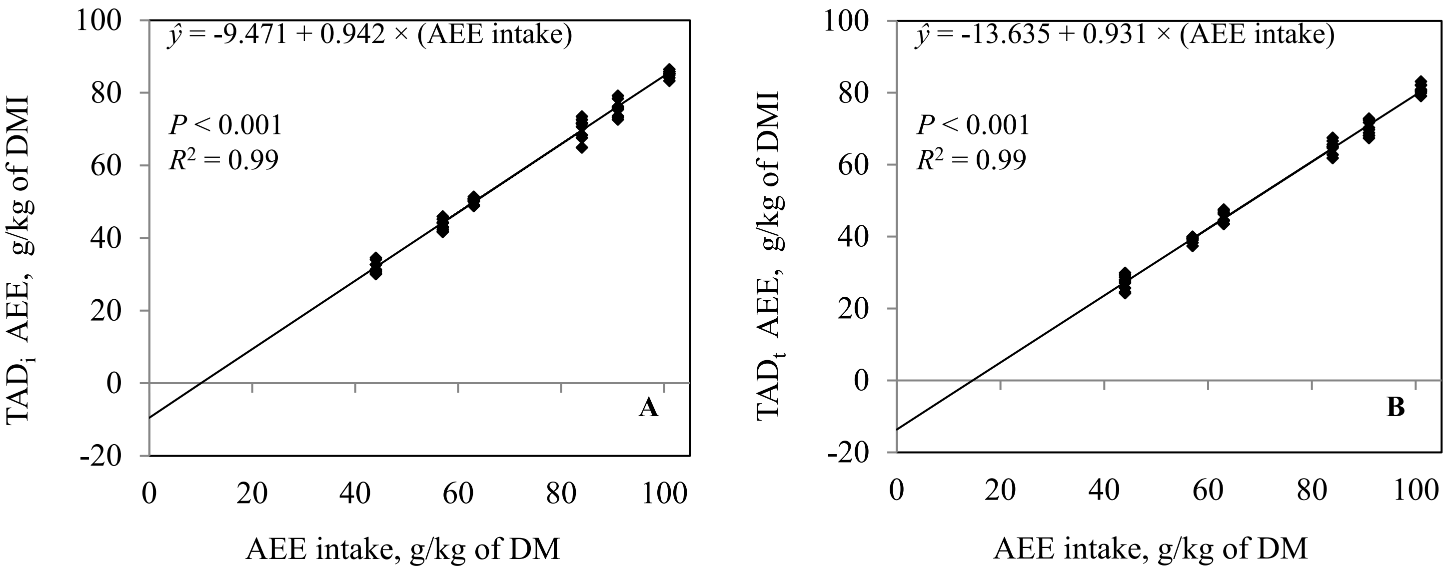


Fig. 3. Estimation of endogenous losses of acid hydrolyzed ether extract (AEE) by regression of total apparently digested AEE at the end of the ileum (TAD$_i$; A) and over the entire intestinal tract (TAD$_t$; B) as a function of dietary AEE intake. The *y*-intercepts are considered the estimated endogenous losses of AEE (g/kg of DMI) and the slope the true digestibility of AEE (Jørgensen et al., 1993).